

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representation of
The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



72706/9

**CORRECTED
VERSION***

PCT

under INTD Numbers (72) and (75) "Inventor, and
Inventor/Applicant (for US only)", the address should
read "41 Marlborough Street, College Park, S.A. 5069
(AU)"

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 6 : A23C 3/00, 7/04, 9/152, A61K 35/20</p>	<p>A1</p>	<p>(11) International Publication Number: WO 97/16977 (43) International Publication Date: 15 May 1997 (15.05.97)</p>
<p>(21) International Application Number: PCT/AU96/00708 (22) International Filing Date: 8 November 1996 (08.11.96) (30) Priority Data: PN 6427 8 November 1995 (08.11.95) AU (71) Applicant (for all designated States except US): NORTHFIELD LABORATORIES PTY. LTD. [AU/AU]; 180 Fosters Road, Oakden, S.A. 5086 (AU). (72) Inventor, and (75) Inventor/Applicant (for US only): SCAMMELL, Antony, William [AU/AU]; 41 Marlborough Street, College Park, S.A. 5069 (AU). (74) Agent: PHILLIPS ORMONDE & FITZPATRICK; 367 Collins Street, Melbourne, VIC 3000 (AU).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.</p>

(54) Title: LIQUID COLOSTRUM FOR DAIRY PRODUCTS

(57) Abstract

The present invention relates to dairy compositions and, more particularly, to dairy compositions containing colostrum. The applicant has discovered that colostrum can be processed so as to reduce the bioburden of liquid colostrum while retaining the biological activity of certain proteins including the immunoglobulin fraction. By this process it is possible to introduce liquid colostrum into pasteurised and unpasteurised dairy products. In a first aspect of the present invention there is provided a process for the preparation of a liquid colostrum product, suitable for inclusion in dairy products, which process includes subjecting colostrum to centrifugation to substantially reduce the microbial content thereof. In a second aspect of the present invention there is provided a method of preparing a dairy composition including undenatured antibodies and/or growth factors which method includes adding to a dairy product a liquid colostrum product.



AU9672706

CT)

(51) International Patent Classification ⁶ : A23C 3/00, 7/04, 9/152, A61K 35/20		A1	(11) International Publication Number: WO 97/16977
			(43) International Publication Date: 15 May 1997 (15.05.97)
(21) International Application Number: PCT/AU96/00708 (22) International Filing Date: 8 November 1996 (08.11.96) (30) Priority Data: PN 6427 8 November 1995 (08.11.95) AU (71) Applicant (for all designated States except US): NORTHFIELD LABORATORIES PTY. LTD. [AU/AU]; 180 Fosters Road, Oakden, S.A. 5086 (AU). (72) Inventor; and (75) Inventor/Applicant (for US only): SCAMMELL, Antony, William [AU/AU]; 41 Marlborough Street, College Park, S.A. 5069 (AU). (74) Agent: PHILLIPS ORMONDE & FITZPATRICK; 367 Collins Street, Melbourne, VIC 3000 (AU).			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.

(54) Title: LIQUID COLOSTRUM FOR DAIRY PRODUCTS

(57) Abstract

The present invention relates to dairy compositions and, more particularly, to dairy compositions containing colostrum. The applicant has discovered that colostrum can be processed so as to reduce the bioburden of liquid colostrum while retaining the biological activity of certain proteins including the immunoglobulin fraction. By this process it is possible to introduce liquid colostrum into pasteurised and unpasteurised dairy products. In a first aspect of the present invention there is provided a process for the preparation of a liquid colostrum product, suitable for inclusion in dairy products, which process includes subjecting colostrum to centrifugation to substantially reduce the microbial content thereof. In a second aspect of the present invention there is provided a method of preparing a dairy composition including undenatured antibodies and/or growth factors which method includes adding to a dairy product a liquid colostrum product.

LIQUID COLOSTRUM FOR DAIRY PRODUCTS

The present invention relates to dairy compositions and, more particularly, to dairy compositions containing colostrum.

5 Raw milk contains between 0% and 1.0% immunoglobulin. However, for safety reasons milk for human consumption is pasteurised - typically at 72°C for 15 seconds or 132°C for at least one second (UHT). Each of these heat treatments is sufficient to denature the immunoglobulin found in raw milk and also to denature certain other milk proteins such as, for example, certain growth
10 factors.

The maximum cfu/mL allowed in milk (Australian Food Standard H1) that has been pasteurised according to the accepted methods is 50,000 cfu/mL. In practice the counts in pasteurised milk at time of manufacture are usually less than 10,000 cfu/mL.

15 Colostrum is the secretion obtained from female mammals just before and for a short period after giving birth. Raw colostrum contains up to 10% immunoglobulin as well as other proteins such as growth factors. However, colostrum is routinely excluded from milk processing due to its very different composition which negatively affects processing efficiency and dairy product
20 quality. Raw colostrum cannot be added to milk products for human consumption due to its bioburden. Colostrum cannot be pasteurised by standard methods without denaturing biologically active proteins such as antibodies. When subjected to heat treatment of greater than approximately 65°C, colostrum will typically undergo severe chemical change leading to the failing of dairy
25 processing equipment.

It is an object of the present invention to overcome, or at least alleviate, one or more of the difficulties or deficiencies associated with the prior art.

The applicant has discovered that colostrum can be processed so as to reduce the bioburden of liquid colostrum while retaining the biological activity of
30 certain proteins including the immunoglobulin fraction. By this process it is possible to introduce liquid colostrum into pasteurised and unpasteurised dairy products including, for example, milk, skim milk, flavoured milks, other modified

milks, yoghurts (natural, flavoured, drinking, frozen), other probiotic formulations, cheese formulations such as cheese sticks, and ice creams.

Accordingly in a first aspect of the present invention there is provided a process for the preparation of a liquid colostrum product, suitable for inclusion in dairy products, which process includes subjecting colostrum to centrifugation to substantially reduce the microbial content thereof.

The colostrum may be freshly harvested colostrum or frozen colostrum. The colostrum may be bovine, ovine or caprine colostrum harvested from freshly calved animals. The colostrum may be harvested using modern milking equipment. Preferably the colostrum is from one or more of the approximately first to tenth milkings after calving. The colostrum may be antibody enriched. For example the colostrum may be from a mammal hyperimmunised against specific pathogens of relevance to human or other diseases.

Specific pathogens which may be used to hyperimmunise the mammal include one or more of:

- | | |
|---------------------------------------------------------------------------|----------------------------------------|
| 1) <i>Cryptosporidium enteridies</i> ,
<i>Cryptosporidium parvum</i> , | 15) <i>Toxoplasma gondii</i> , |
| 2) <i>Isospora belli</i> , | 16) <i>Clostridium</i> sp., |
| 3) <i>Giardia</i> sp, | 17) <i>Campylobacter jejuni/coli</i> , |
| 4) <i>Cytomegalovirus</i> sp, | 18) <i>Entamoeba histolytica</i> , |
| 5) <i>Salmonella</i> sp, | 19) Human immunodeficiency virus |
| 6) <i>Shigella</i> sp, | 20) <i>Helicobacter pylori</i> , |
| 7) <i>Candida</i> sp, | 21) <i>Cholera</i> sp., |
| 8) <i>Rotavirus</i> , | 22) Respiratory syncytial virus |
| 9) <i>Blastocystis hominis</i> , | 23) <i>Haemophilus influenzae</i> |
| 10) <i>Herpes simplex virus</i> , | 24) <i>Astrovirus</i> |
| 11) Enterotoxigenic <i>E. Coli</i> , | 25) <i>Adenovirus</i> |
| 12) <i>Aeromonas</i> sp, | 26) <i>Mycobacterium</i> spp. |
| 13) <i>Mycobacterium Avium</i> intracellular, | 27) <i>Calici virus</i> |
| 14) <i>Yersinia enterocolitica</i> , | 28) <i>Toro virus</i> |

The colostrum may be from a mammal hyperimmunised against specific proteins or enzymes such that the specific antibodies produced are of relevance in the prevention or treatment of human diseases or metabolic disorders.

5 The bacteria-removing centrifugation may be performed at a speed of approximately 7000-8000 rpm, preferably approximately 7400 rpm, to generate approximately 12,000 g at approximately 1600 L per hour. An Alfa Laval D3187M-60 Bactofuge has been found to be suitable.

10 Ideally, it is preferable that the centrifugation is performed by controlling throughput and thereby residence time of the colostrum such that the microbial content is reduced to less than 500 organisms per ml, preferably 200 organisms per ml, most preferably less than 50 organisms per ml.

Preferably, the centrifugation is conducted at a temperature of approximately 50°C to approximately 64°C. More preferably, the centrifugation is conducted at a temperature of approximately 55°C to approximately 63°C.

15 Following centrifugation, the colostrum may be chilled to below approximately 10°C, preferably to below approximately 4°C.

In a preferred form, the process of this aspect of the invention may include the preliminary step of separating the colostrum. Preferably the colostrum is separated into a light phase (cream) and a heavy phase (skim). Preferably the
20 light phase is discarded and the heavy phase is processed according to the present invention.

Any suitable separation apparatus may be used. A preferred separation apparatus is a self desludging type apparatus.

25 Preferably the colostrum is pre-heated to a temperature of approximately 50°C to approximately 64°C, more preferably approximately 55°C to approximately 63°C, for a period of approximately 3-60 minutes, more preferably approximately 30 minutes, prior to the separation step. Any suitable means may be used to preheat the colostrum, for example a plate heat exchanger.

30 It will be understood that the steps of the process of this aspect of the present invention may take place in any suitable order. Other steps may also be included in the process of the present invention. For example in preferred embodiment of the process casein is removed from the colostrum by precipitation.

Preferably this step takes place after the colostrum has first been subjected to a separation step and the light phase has been discarded, and then subjected to centrifugation according to the present invention.

5 In a further preferred embodiment of the process the colostrum is concentrated by means of ultrafiltration and/or evaporation and/or reverse osmosis. Preferably this step takes place after either the centrifugation or, if relevant, after the casein removal.

In a still further preferred embodiment of the process the colostrum is sterile filtered. Preferably this step takes place after the casein removal.

10 The method according to the present invention gives results that are consistently better than the results gained with standard pasteurisation and allows for the addition of colostrum to pasteurised dairy products and/or the processing of dairy products including a colostrum component with little or no diminution of the shelf life/stability expected with normal dairy products. For example,
15 colostrum may be added to dairy products including raw or pasteurised whole milk, skim milk, or modified milk products including flavoured milks, and the resulting mixture processed as described above.

Applicant has found that the total microbial count in liquid colostrum treated according to the process of the present invention is reduced by at least
20 approximately 80%, preferably at least approximately 90%, more preferably at least approximately 95%. For example, a combination of gentle heat treatment at 55°C and bactofugation typically renders a minimum 99% reduction in total microbial count. Moreover, a substantial proportion of the antibodies and/or growth factors in the liquid colostrum produced according to the method of the
25 present invention remain undenatured following the treatment.

Because of the high concentration of immunoglobulin in the fresh, processed colostrum, only a relatively small proportion of colostrum is required to be added to milk or dairy products to bring the resulting product up to the level of antibodies normally found in pre-processed milk, eg., approximately 10%
30 colostrum processed according to the invention will typically provide 100% as much immunoglobulin or more as pre-processed milk.

Due to the economy and effectiveness of this approach it is now possible to

safely provide dairy produce which contains undenatured antibodies and growth factors. The undenatured immunoglobulins may be targeted against specific human diseases or metabolic disorders by hyperimmunising the mammal, providing a very effective palatable form of therapy.

5 Accordingly, in a second aspect of the present invention there is provided a method of preparing a dairy composition including undenatured antibodies and/or growth factors which method includes adding to a dairy product a liquid colostrum product.

10 Preferably the liquid colostrum product is from a bovine, ovine or caprine source. Preferably the liquid colostrum product is produced by the process of the present invention. The liquid colostrum product may be added to the dairy product at any time. Preferably, the addition occurs after the dairy product has been pasteurised.

15 The dairy product may be of any suitable type and includes pasteurised and unpasteurised dairy products such as milk, for example raw or pasteurised full-cream, skim, modified, flavoured milk; yoghurt, for example natural, flavoured, frozen or drinking yoghurt; tonics and sports drinks; and other dairy products such as custards, cheese and cottage cheese formulations, and ice creams.

20 Preferably the colostrum is added in an amount of approximately 1% w/w to approximately 50% w/w, more preferably approximately 2% w/w to approximately 20% w/w, most preferably approximately 5% w/w to approximately 10% w/w.

25 In this manner it is possible to provide a milk or other dairy product containing a measurable quantity of undenatured antibodies, preferably at a concentration similar to or greater than that found in raw milk prior to a normal pasteurisation process (eg. approximately 0.2% IgG). Alternatively or in addition the milk or other dairy product may contain undenatured growth factors.

In a still further aspect of the present invention there is provided a dairy composition for human consumption including liquid colostrum that has not been subjected to a drying process.

30 Preferably, the liquid colostrum product is from a bovine, ovine or caprine source. Preferably the liquid colostrum product is produced by the process of the present invention.

The dairy product may be of any suitable type and includes pasteurised and unpasteurised dairy products such as milk, for example raw or pasteurised full-cream, skim, modified, flavoured milk; yoghurt, for example natural, flavoured, frozen or drinking yoghurt; tonics and sports drinks; and other dairy products such as custards, cheese and cottage cheese formulations, and ice creams.

Preferably, the dairy composition includes approximately 1% w/w to approximately 50% w/w, more preferably approximately 2% w/w to approximately 20% w/w, most preferably approximately 5% w/w to approximately 10% w/w of liquid colostrum.

In this manner it is possible to provide a milk or other dairy product containing a measurable quantity of undenatured antibodies, preferably at a concentration similar to or greater than that found in raw milk prior to a normal pasteurisation process (eg. approximately 0.2% IgG w/w). Alternatively or in addition the milk or other dairy product may contain undenatured growth factors.

The present invention will now be more fully described with reference to the accompanying Examples. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

Example 1 - Centrifugation of Colostrum

Materials: skimmed colostrum
whole colostrum

Apparatus: Alfa Laval D-3187M-60 Bacterial centrifuge

SUMMARY

This study was designed to test the suitability of the Alfa Laval D3187M-60 at reducing bacterial loads in colostrum. The equipment was installed in-line (between Past I and UFS-2) to reduce total bacterial counts with the aim of reliably producing a product with less than 1000 organisms/gram.

PURPOSE

To evaluate the suitability of the Alfa Laval D3187M-60 at reducing total bacterial load in colostrum.

PROCEDURE

Bactofuge D3187M-60 was installed and commissioned on H₂O. Flow rates were set at: inlet = 672 lph

outlet = 600 lph

5 bactofugate = 72 lph

i) Skimmed colostrum

Skim colostrum was heated to 55°C for 30 minutes (not pasteurised) and passed through the bactofuge using the parameters outlined above.

N.B. heating to 55°C reduced bacterial count from 2.65×10^7 to 4.9×10^6 or

10 81.5% reduction, which is not suitable for human consumption.

ii) Whole colostrum batch 2B02

Whole colostrum was processed as a complete batch and various microbiological samples taken.

iii) Whole colostrum batch 2B03

15 Whole colostrum was processed as a complete batch through a powdered stage.

RESULTS

i) Skimmed Colostrum

Raw colostrum	2.65×10^7 cfu/mL
Heat treated skim (55°C, 3 min)	4.9×10^6 (81.5% reduction)
Centrifuged skim	1.86×10^3 (99.96% reduction)
Total reduction	99.99%

20

ii) BATCH 2B02

Raw colostrum	1.22×10^4 cfu/mL
Heat treated (60°C, 30 min)	6.65×10^2 (94.5% reduction)
Centrifuged skim	16 (97.6% reduction)
Total reduction	99.9%

iii) BATCH 2B03

Raw colostrum	1.42×10^5 cfu/mL
Heat treated (63°C, 30 min)	3.2×10^2 (99.8% reduction)
Centrifuged skim	33 (89.7% reduction)
Total reduction	99.98%

DISCUSSION

The results indicate that the Alfa Laval DM-3187M-60 Bacterial Centrifuge is effective at removing up to approximately 99% of heat resistant organisms (ie. those organisms surviving heat treatment), providing a total reduction in bioburden from raw colostrum of at least 99.9%.

With a good quality colostrum it is possible to produce good quality concentrate (<100 cfu/mL).

10 CONCLUSIONS

The Alfa Laval D-3187M-60 Bacterial Centrifuge is effective at removing organisms from colostrum to an extent that it is acceptable for inclusion in dairy products such as milk.

15 Example 2 - Reduction of colony forming units after bacterial centrifugation

Samples of colostrum were collected. The colostrum was sampled and then treated by firstly separating the colostrum into a light phase (cream) and a heavy phase (skim) before bacterial centrifugation. Samples of each batch (treated and raw) were removed at various stages, including before and after separation into light (cream) and heavy (skim) phases, and before and after bacterial centrifugation. Bacterial counts measured as colony forming units (CFU)/mL were assessed to determine overall reduction of CFU's.

PROCEDURE

Bactofuge D3187M-60 was installed and commissioned on H₂O. Flow rates were set at approximately: inlet = 1600 lph
 outlet = 1440 lph
 bactofugate = 160 lph

i) Skimmed colostrum

Skim colostrum was heated to 55°C for 3 minutes (not pasteurised) and passed through the bactofuge using the parameters outlined above.

RESULT

- 5 Thirty seven samples of colostrum were processed as outlined above. After bacterial centrifugation the average CFU/ml was 44.97 CFU/ml which represented a 99.86% overall reduction of CFU's from raw colostrum to the processed colostrum product.

CONCLUSION

- 10 Clearly the combination of skimmed colostrum heated to 55°C and centrifuged at the 1600 Lph flowthrough rate is an effective means of reducing bacterial load whilst reducing the incidence of denaturing the biologically active proteins by exposure to high temperatures.

15 Example 3: Dairy CompositionPROCEDURE

- The colostrum was processed as described in Example 2. Two separate experiments were conducted. The first was from batch 6F01 which was a batch of low litre colostrum of all failed materials, such that its original bacteriology was very dirty prior to bactofugation. The second was from batch 6F03 which was high titre colostrum of all "A" grade materials such that its original bacteriology was very clean.

The figures for the raw and bacterial centrifuged samples are given in Table 1 below.

Sample	Total Count	Coliform	Staphylococcus
6F01 Raw	500,00	670	380,000
6F01 Bactofuged	11	0	0
6F03 Raw	1,400	4	10
6F03 Bactofuged	5	0	0

Table 1

RESULTS

The results for the bacterial centrifuged colostrum from batch 6F01 when added to whole milk and skim milk at 10% are given in Table 2 and compared with milk and colostrum controls:

5

Sample Time	Neat Colostrum	10% Col./ Whole Milk	10% Col./ Skim Milk	Whole Milk Control	Skim Milk Control
Time 0	11	1,100	1,300	1,400	1,400
2 days	15	1,200	1,200	1,100	1,400
4 days	16	1,200	1,500	1,300	1,600
6 days	13	1,500	1,400	1,400	1,400
8 days	12	1,200	1,400	1,200	1,600
10 days	18	1,100	1,300	1,200	1,400
12 days	7	1,000	1,200	1,200	1,600
14 days	9	1,000	1,600	1,100	1,100

Table 2

All samples in experiment 1 showed no changes to bacterial load over the test period. Furthermore the neat colostrum and the 10% colostrum in full cream milk sample (samples 1 and 2) continued to be tested. Sample 2 took 30 days to reach 58,000 cfu/mL and sample 1 showed no growth at the 46 day mark.

10

The results for the bacterial centrifuge bactofuged colostrum from batch 6F03 when added to whole milk and skim milk at 10% are given in Table 3 and compared with milk and colostrum controls.

15

Sample Time	Neat Colostrum	10% Col./ Whole Milk	10% Col./ Skim Milk	Whole Milk Control	Skim Milk Control
Time 0	5	1,600	1,500	1,700	1,700
2 days	7	1,400	1,500	1,500	1,400
4 days	9	1,100	1,500	1,400	1,600
6 days	9	1,400	1,300	1,500	1,500
8 days	4	1,200	1,200	1,500	1,600
10 days	13	7,700	6,200	5,100	15,300
12 days	500	180,000	140,000	200,000	400,000
14 days	74,000	32,000,000	27,000,000	45,000,000	61,000,000

Table 3

5 Samples in experiment 2 showed no difference in bacterial loading between dairy compositions with colostrum processed and those without.

Considering both experiments together it can be seen that the addition of colostrum to full cream or skimmed milk has no affect on the shelf life of those products.

10 Example 4 - Stability of Antibodies in Dairy Compositions

AIM

The aim of the study was to assess the stability of colostral antibodies at 4°C for 14 days.

MATERIAL AND METHODS

15 Two batches of colostrum 6F01 and 6F03, including high and low titre of antibodies was used for this study. From each batch 10% of colostrum was mixed with whole milk as well as with skim milk. Samples were withdrawn on day 0, 2, 4, 6, 8, 10, 12, 14 from 4°C and frozen -20°C until assayed.

20 The experiment was performed using standard SOP10.01 and each sample was tested three times.

RESULTS

The two tables below showing the result of rotavirus antibodies titre over 14

days, in two batches of colostrum.

BATCH 6 F03

TABLE 4

Day	Neat Colostrum	10% Colostrum in Whole Milk	10% Colostrum in Skim Milk
0	48960	4841	4874
2	56378	4544	3903
4	50937	4841	4348
6	56898	5008	5415
8	61612	5063	5750
10	61612	5154	4829
12	55024	3927	5803
14	66839	5063	5334

5

BATCH 6 F01

TABLE 5

Day	Neat Colostrum	10% Colostrum in Whole Milk	10% Colostrum in Skim Milk
0	3263	491	472
2	2979	468	458
4	2737	468	485
6	3062	493	430
8	2840	464	462
10	3255	549	463
12	3115	463	459
14	3179	541	465

DISCUSSION

10

Table 6 shows the summary of the results from this study.

TABLE 6: Results of testing rotavirus antibodies in high and low titre milk samples by ELISA.

	SAMPLE	MEAN	SDEV	COVAR	T-TEST
POSITIVE BATCH 6F03	1	57282	5908	10%	0.26
	2	4805	403	8%	0.99
	3	5040	657	10%	0.33
NEGATIVE BATCH 6F01	1	3054	191	6%	0.72
	2	492	37	7%	0.57
	3	462	16	10%	0.96

- 5 SAMPLE 1 = neat
 SAMPLE 2 = 10% colostrum in whole milk
 SAMPLE 3 = 10% colostrum in skim milk

DISCUSSION

- 10 The results of this study indicate that rotavirus antibodies measured by ELISA in colostrum are considerably stable at 4°C over 14 days in the dairy composition according to the invention.

CONCLUSION

- 15 This study shows that colostrum prepared according to the invention has very stable rotavirus antibodies and can be stored in the fridge for period of at least 2 weeks with no detrimental effects to the rotavirus antibody titre.

Example 5 - Taste testing

PURPOSE

- 20 To determine on a small scale if subjects could detect a difference in taste and preference for milk preparations containing various concentrations of freshly processed liquid colostrum.

MATERIALS

Homogenised, full cream milk

Skimmer milk

Iced Coffee milk

Batch 5F01 colostrum obtained directly after separation and bactofugation.

Numbered cups.

5 METHODS

Three experiments were carried out to determine the effect colostrum had on the taste of 3 types of milk.

Experiment one

Colostrum was mixed with Iced coffee milk product in the proportions:

- 10 0%, 2%, 8%, 20%. The different products were put into numbered cups, and subjects asked to taste each product and rank the numbered cups in order of taste preference.

<u>Concentration %</u>	<u>Cup No.</u>
0	3
2	1
8	2
20	4

15 RESULTS:

<u>Person</u>	<u>Ranking</u>				<u>Comments</u>
AS	3	4	2	1	2 creamiest, 1 more like skim
GP	3	4	2	1	3 is the iced coffee product, 4 is most like real coffee
RP	1	4	2=	3=	did not like 2 or 3
RR	=	=	=	=	no difference
CC	=	=	=	=	all nice
JP	=	=	=	=	all OK

The results from experiment one indicate that:

1. Subjects were not able to detect taste difference to ice coffee milk when separated bactofuged colostrum from batch 5F01 was added up to 20% w/w.

2. Cups 3 and 4 were preferred by two subjects. These products respectively contained 0% and 20% colostrum, again indicating that the addition of colostrum does not significantly affect taste preference.

Experiment two

- 5 Colostrum was mixed with homogenised full cream milk product in the proportions:

0%, 2%, 8%, 20%. The different products were put into numbered cups, and subjects asked to taste each product and rank the numbered cups in order of taste preference.

10

<u>Concentration %</u>	<u>Cup No.</u>
0	2
2	4
8	1
20	3

RESULTS:

<u>Person</u>	<u>Ranking</u>				<u>Comments</u>
AS	3	2	=	=	On first tasting, 3 seemed best. On second tasting, 2 seemed best
PW	3	1	2	4	Not really much difference
RP	=	=	=	=	Couldn't tell, all tasted bad.
BC	2	1	4	3	
MS	2	3	4	1	All OK, can't tell much difference
JT	1=	4=	3=	2	Only 2 seemed worse

The results from experiment two indicate that:

- 15 1. Subjects were not able to detect taste difference to milk when separated
bactofuged colostrum from batch 5FO1 was added up to 20% w/w.
2. Cups 2 and 3 were preferred by two subjects each. These products
respectively contained 0% and 20% colostrum, again indicating that the
addition of colostrum does not significantly affect taste preference.
- 20 3. One person ranked the 20% colostrum worst, while one ranked the 0%

colostrum worst. The person ranking the 20% colostrum worst ranked the normal product best. This was the only person to get close to ranking taste preferences according to colostrum content.

Experiment three

- 5 Colostrum was mixed with skimmed milk product in the proportions: 0%, 4%, 16%, 40%. The different products were put into numbered cups, and subjects asked to taste each product and rank the numbered cups in order of taste preference.

<u>Concentration %</u>	<u>Cup No.</u>
0	1
4	2
16	3
40	4

10

RESULTS:

<u>Person</u>	<u>Ranking</u>				<u>Comments</u>
AS	1	2	3	4	Colour difference was noticeable, taste OK.
PW	=	=	=	3	3 was least liked
RP	2	3	1	4	
BC	1	3	2	4	
MSp	2	3	4	1	All OK, can't tell which is milk, 4 is richer, 2 is creamier than 1.
MS	1	2	4	3	

The results from experiment three indicate that:

- 15
1. When colostrum from batch 5F01 was added to skimmed milk, at the higher concentrations, a difference was noticeable, both in colour and mouth feel.
 2. Cup 4 (40% colostrum) was ranked bottom, taste was acceptable, just noticeably different.

CONCLUSION

Overall the study indicates that fresh, skim, bactofuged colostrum similar to that produced in batch 5F01 should be an acceptable additive to white milk and flavoured milk in the proportion intended (approximately 20% or less). Only when
5 added to skim at 16% or more is there likely to be a noticeable difference.

Finally, it is to be understood that various alterations, modifications and/or additions may be made without departing from the spirit of the present invention as outlined herein.

CLAIMS:

1. A process for the preparation of a liquid colostrum product, suitable for inclusion in dairy products, which process includes subjecting colostrum to centrifugation to substantially reduce the microbial content thereof.
2. A process according to claim 1 wherein the centrifugation is performed by controlling throughput and residence time of the colostrum during centrifugation such that microbial content is reduced to less than 1000 organisms per mL.
3. A process according to claim 2 wherein the centrifugation is performed by controlling throughput and thereby residence time of the colostrum during centrifugation such that microbial content is reduced to less than 200 organisms per mL.
4. A process according to claim 2 wherein the centrifugation is performed by controlling throughput and thereby residence time of the colostrum during centrifugation such that microbial content is reduced to less than 50 organisms per mL.
5. A process according to any one of claims 1 to 4 wherein the centrifugation is performed to generate 12,500 g.
6. A process according to any one of claims 1 to 5 wherein the centrifugation is conducted at a temperature of approximately 50°C to approximately 64°C.
7. A process according to claim 6 wherein the centrifugation is conducted at a temperature of approximately 55° C to approximately 63° C.
8. A process according to anyone of claims 1 to 7 wherein the colostrum is chilled to below 10°C following centrifugation.
9. A process according to claim 8 wherein the colostrum is chilled to below approximately 4°C.
10. A process according to any one of claims 1 to 9 including a preliminary step of separating the colostrum into a light phase and a heavy phase and wherein the heavy phase is centrifuged.
11. A process according to any one of claims 1 to 10 including a preliminary step of preheating the colostrum to a temperature of approximately 50°C to approximately 64°C prior to centrifugation.

12. A process according to claim 11 wherein the colostrum is preheated to approximately 63°C.
13. A process according to claim 11 or 12 wherein the colostrum is preheated for approximately 3 to 60 minutes.
- 5 14. A process according to claim 13 wherein the colostrum is preheated for approximately 30 minutes.
15. A process according to any one of claims 1 to 14 including removing casein from the colostrum prior to centrifugation.
16. A process according to claim 15 wherein the casein is removed after
10 separation of the colostrum into a light phase and a heavy phase.
17. A process according to any one of claims 1 to 16 including concentrating the colostrum either after centrifugation or after casein removal.
18. A process according to claim 17 wherein the colostrum is concentrated by means of ultrafiltration and/or evaporation and/or reverse osmosis.
- 15 19. A process according to any one of claims 1 to 18 wherein the colostrum is sterile filtered.
20. A process according to any one of claims 1 to 19 wherein the colostrum is freshly harvested or frozen.
21. A process according to any one of claims 1 to 20 wherein the colostrum is
20 bovine, ovine or caprine colostrum.
22. A process according to any one of claims 1 to 21 wherein the colostrum is obtained from a mammal hyperimmunised against a pathogen.
23. A process according to claim 22 wherein the pathogen includes one or more of the pathogens selected from the group including:
- | | | |
|----|----------------------------------------|----------------------------------------|
| 25 | 1) <i>Cryptosporidium enteridies</i> , | 15) <i>Toxoplasma gondii</i> , |
| | <i>Cryptosporidium parvum</i> , | |
| | 2) <i>Isoporo belli</i> , | 16) <i>Clostridium</i> sp., |
| | 3) <i>Giardia</i> sp, | 17) <i>Campylobacter jejuni/coli</i> , |
| | 4) <i>Cytomegalovirus</i> sp, | 18) <i>Entamoeba histolytica</i> . |
| 30 | 5) <i>Salmonella</i> sp, | 19) Human immunodeficiency virus |
| | 6) <i>Shigella</i> sp, | 20) <i>Helicobacter pylori</i> , |

- | | | | |
|-------|-----------------------------------|-----|----------------------------|
| 7) | Candida sp, | 21) | Cholera sp., |
| 8) | Rotavirus, | 22) | Respiratorysyncitial virus |
| 9) | Blastocystis hominis, | 23) | Heamophilus influenzae |
| 10) | Herpes simplex virus, | 24) | Astrovirus |
| 5 11) | Enterotoxigenic E. Coli, | 25) | Adenovirus |
| 12) | Aeromonas sp, | 26) | Mycobacterium spp. |
| 13) | Mycobacterium Avium intracellular | 27) | Calici virus |
| 14) | Yersinia entero-colitica, | 28) | Toro virus |

10 24. A process according to any one of claims 1 to 23 which excludes any drying processes.

25. A liquid colostrum product prepared by a process according to any one of claims 1 to 24.

15 26. A method of preparing a dairy composition including undenatured antibodies and/or growth factors which method includes adding to a dairy product a liquid colostrum product.

27. A method according to claim 26 wherein the liquid colostrum product is added after pasteurisation of the dairy product.

20 28. A method according to claim 26 wherein the liquid colostrum product is a product according to claim 25.

29. A method according to any one of claims 26 to 28 wherein the liquid colostrum product is added in an amount of approximately 1 % w/w to approximately 50% w/w.

25 30. A method according to any one of claims 26 to 29 wherein the colostrum is added in an amount of approximately 2% w/w to approximately 20% w/w.

31. A method according to any one of claims 26 to 30 wherein the colostrum is added in an amount of approximately 5% w/w to approximately 10% w/w.

32. A dairy composition prepared by the method according to any one of claims 26 to 31.

30 33. A dairy composition according to claim 32 including a measurable quantity of undenatured antibodies and/or growth factors at a concentration similar to or

greater than that found in raw dairy products prior to normal pasteurisation processes.

34. A dairy composition according to claims 32 or 33 including a dairy product selected from the group including pasteurised and unpasteurised dairy products including milk which is raw or pasteurised, full-cream, skim, modified or flavoured milk; yoghurt, including natural, flavoured, frozen or drinking yoghurt; tonics and sports drinks; and other dairy products including custards, cheese and cottage cheese formulations, and ice creams.

35. A method of preventing and treating a human disease or metabolic disorder including administering an effective amount of a dairy composition according to any one of claims 32 to 34.

36. A method of preventing and treating a human disease or metabolic disorder caused by any one or more pathogens selected from the group including:

- | | | | |
|----|---------------------------------------------------------------------------|-----|------------------------------------|
| 15 | 1) <i>Cryptosporidium enteridies</i> ,
<i>Cryptosporidium parvum</i> , | 15) | <i>Toxoplasma gondii</i> , |
| | 2) <i>Isoporo belli</i> , | 16) | <i>Clostridium</i> sp., |
| | 3) <i>Giardia</i> sp, | 17) | <i>Campylobacter jejuni/coli</i> , |
| | 4) <i>Cytomegalovirus</i> sp, | 18) | <i>Entamoeba histolytica</i> , |
| 20 | 5) <i>Salmonella</i> sp, | 19) | Human immunodeficiency
virus |
| | 6) <i>Shigella</i> sp, | 20) | <i>Helicobacter pylori</i> , |
| | 7) <i>Candida</i> sp, | 21) | <i>Cholera</i> sp., |
| | 8) <i>Rotavirus</i> , | 22) | Respiratory syncytial virus |
| 25 | 9) <i>Blastocystis hominis</i> , | 23) | <i>Haemophilus influenzae</i> |
| | 10) <i>Herpes simplex virus</i> , | 24) | <i>Astrovirus</i> |
| | 11) Enterotoxigenic <i>E. Coli</i> , | 25) | <i>Adenovirus</i> |
| | 12) <i>Aeromonas</i> sp, | 26) | <i>Mycobacterium</i> spp. |
| | 13) <i>Mycobacterium Avium</i> intracellular, | 27) | Calici virus |
| 30 | 14) <i>Yersinia enterocolitica</i> , | 28) | Toro virus |

said method including administering an effective amount of a dairy composition according to any one of claims 32 to 34.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00708

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: A23C 3/00 7/04 9/152 A61K 35/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC : A23C 3/00 7/04 9/152 A61K 35/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU : IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPAT : COLOSTRUM and IPC as above

JAPIO : as for WPAT FSTA: COLOSTRUM and (CENTRIFUG: or BACTOFUG:)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5143727 A (EBINA) 1 September 1992 Examples 1 and 3	1, 20-25
X	AU 58339/94 A (HORSE VITALITY LTD) 4 August 1994 Page 1 lines 9-14; page 4 lines 7-9; examples 1-3	26, 27, 29-36
X	AU 39340/89 A (BORODY and COOPER) 8 February 1990 Page 4 lines 24-32; page 5 lines 1-14; example	26, 27, 29-36

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 December 1996

Date of mailing of the international search report

19 DEC 1996

Name and mailing address of the ISA/AU
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
PO BOX 200
WODEN ACT 2606
AUSTRALIA Facsimile No.: (06) 285 3929

Authorized officer

N.L. KING

Telephone No.: (06) 283 2150

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00788

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	AU 63136/94 A (MINISTER OF PRIMARY INDUSTRIES) 7 December 1995 Example 1	1, 25
A	US 4342747 A (LIOTET and ASSIER) 3 August 1982 Examples 1-5	26, 32

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00708

This Annex lists the known "A" publication level patent family members relating to the parent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
US	5143727	AT EP	20309 46909	CA JP	1175352 57042629	DE JP	3174807 4043888
AU	58339/94	CA WO	2154652 9416675	EP IT	675708 1263819	JP ZA	8505630 9400507
AU	39340/89	AU	629559	CA	1337265		
AU	63136/94	AU	668033	NZ	260568		
US	4342747	BE FR JP	883935 2460135 56053612	CH GB JP	644269 2052979 2001127	DE IT NL	3024623 1130487 8003802
END OF ANNEX							